FLOCKTYPE® recNDV

ELISA Test Kit to Detect Antibodies to Newcastle Disease Virus in the Chicken

Labor Diagnostik
Leipzig

Instructions for Use

In vitro Diagnostic Kit for Veterinary Medicine,
registered in accordance with §17c of the Law on Animal Diseases
Registration No. FLI-B 394

Applications

The FLOCKTYPE® recNDV is an enzyme immunoassay (ELISA) in the microtitre plate format for the detection of antibodies to the Newcastle Disease Virus (NDV) in chicken serum and plasma.

General Information

The FLOCKTYPE® product line is a new generation of test kits for the serological monitoring of poultry flocks. The use of recombinant antigens guarantees that all FLOCKTYPE® ELISA kits can be well standardised.

The antigen used in the FLOCKTYPE® recNDV ELISA is the nucleocapsid protein of the Newcastle Disease Virus prepared by recombinant technology. This protein is a major structural protein of the virus. The FLOCKTYPE® recNDV ELISA in combination with the FlockSoft™ software is capable of detecting the antibody titre in the chicken induced by vaccination or by natural infections and of quantitatively depicting the results.

Description of the Test Principle

The microtitre plate is coated with recombinant NDV antigen. During sample incubation, NDV-specific antibodies bind to the immobilised antigen; unbound material is removed by rinsing. The anti-IgY-HRP conjugate detects serum antibodies bound to the antigen. Unbound conjugate is rinsed out. The colour reaction is started by adding the substrate solution and stopped after 10 minutes. The optical density (OD) is measured in a spectrophotometer; the OD values correlate with the concentration of anti-NDV antibodies in the sample.

Reagents

		2 plate kit	5 plate kit
1.	Test Plate, contains 12 microtitre strips with 8 wells each or Test Plate, microtitre plate with 96 wells, coated with non-infectious recombinant NDV antigen	2	5
2.	Wash Buffer (10×), buffer solution with Tween and preservative	1x 125 ml	2x 125 ml
3.	Dilution Buffer, buffer with protein and preservative	1x 125 ml	2x 125 ml
4.	Positive Control, NDV-reactive chicken serum in buffer with protein stabilizers and preservative, ready-to-use	1x 2.5 ml	1x 2.5 ml
5.	Negative Control, NDV-negative chicken serum in buffer with protein stabilizers and preservative, ready-to-use	1x 2.5 ml	1x 2.5 ml
6.	Anti-IgY-HRP, rabbit anti-chicken IgY-horseradish peroxidase conjugat in buffer with protein stabilisers and preservatives, ready-to-use	1x 24 ml	1x 60 ml
7.	TMB, Tetramethylbenzidine Substrate Solution, ready-to-use	1x 24 ml	1x 60 ml
8.	Stop Solution, 0.5 M sulfuric acid, ready-to-use, corrosive!	1x 24 ml	1x 60 ml

Additional Material and Equipment Required

Beakers, measuring cylinders, analytical pipettes, multichannel pipettes, disposable pipette tips, pipetting troughs, microtitre plate spectrophotometer, tubes or plates for diluting the samples, distilled water

Precautions and Warnings

Store the reagents at 2-8 °C and only bring them to room temperature (18-25 °C) immediately before use. Store the remaining test strips in the re-sealed pack with desiccant at 2-8 °C until next use. The test strips can be stored at least for 6 weeks after opening the plate pack.

The test should be performed by persons qualified for laboratory work. Store the TMB Substrate Solution in the dark and do not expose it to intense light or to sunlight during the performance of the test. The components of the test kit should not be contaminated or mixed with components from other batches. Do not use the components of the test kit past expiration date. Water from ion-exchange systems used for diluting the buffer concentrate may interfere with the assay if not pure enough. Water of the quality of double distilled water or highly purified water (Milli-Q) is suitable.

To guarantee the precision of the results, it is absolutely essential to follow the usual precautions for ELISA procedures, including the use of clean glass devices, careful pipetting and rinsing during the test, and strict adherence to the indicated incubation times. The kit contains hazardous substances (Sulphuric acid). All sample residues and objects which have come into contact with samples must be decontaminated or disposed of as potentially infectious material.

Preparation of Reagents and Samples

Wash Buffer:

Wash Buffer (10x), bottle 2, dilute 1:10 with distilled water,
e.g., for one Test Plate dilute 25 ml Wash Buffer (10x) in 225 ml distilled water and mix.

Serum, plasma:

Before using the samples in the assay, dilute them 1:500 with Dilution Buffer, e.g. 1 µl sample is diluted in 499 µl Dilution Buffer and mixed. Be sure to change pipette tips for each sample. Controls are ready-to-use, do not dilute them.
Alternatively, serum/plasma samples can be diluted from a pre-dilution (1:50 in Dilution Buffer) directly in the Test Plate (see Test Procedure, 1. Filling the Test Plate).

Test Procedure

Bring all reagents to room temperature (18-25 °C) before use and mix well.

1. Filling the Test Plate:

   Record the positions of the controls and samples in a test protocol, e.g. Negative Control (NC) = A1/B1; Positive Control (PC) = C1/D1; other positions of the samples.

   Pipette 100 µl of each of the ready-to-use Negative and Positive Control (in duplicates) and the 1:500 diluted samples into the Test Plate wells. Alternatively, pipette 90 µl of Dilution Buffer in each well and add 10 µl of the 1:50 pre-diluted sample. Mix well.
   Cover the Test Plate.

   Template for FLOCKTYPE® recNDV ELISA

	1	2	3	4	5	6	7	8	9	10	11	12
A	NC	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77	S85
B	NC	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78	S86
C	PC	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79	S87
D	PC	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80	S88
E	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73	S81	S89
F	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74	S82	S90
G	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75	S83	S91
H	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76	S84	S92

2. Incubate for 30 min at room temperature and then empty the wells by aspiration or tapping.

3. Rinse each well 3x with 300 µl of prepared Wash Buffer. Remove the buffer after each rinse.

4. Add 100 µl ready-to-use anti-IgY-HRP to each well.

5. Incubate for 30 min at room temperature and then empty the wells by aspiration or tapping.

6. Rinse each well 3x with 300 µl of prepared Wash Buffer. Remove the buffer after each rinse.

7. Add 100 µl TMB Substrate Solution to each well.

8. Incubate for 10 min at room temperature in the dark.

9. Stop the reaction by adding 100 µl Stop Solution per well.

10. Calibrate the spectrophotometer against air as blank. Measure the optical density (OD) in the spectrophotometer at 450 nm immediately or within 20 min after stopping the reaction. Measuring at a reference wavelength (620-650 nm) is optional.

Test Validation

For the assay to be valid the mean value (MV) of the measured OD for the Positive Control must be ≥ 0.7; the mean value (MV) of the measured OD for the Negative Control must be ≤ 0.2.

Calculation

1. Calculate the mean values (MV) of the measured OD for the Negative Control (NC) and the Positive Control (PC).

2. Subtract the mean OD of NC from the OD of the sample and from the mean OD of PC.

3. The ratio sample to mean PC is calculated according to the following equation:

$$\text{S/P ratio} = \frac{OD_{sample} - OD(MV)_{NC}}{OD(MV)_{PC} - OD(MV)_{NC}}$$

4. Endpoint titres are calculated from the S/P ratio at a 1:500 dilution using the following equation:

$$\text{Log}_{10}\text{Titre} = 1.54\ (\text{Log}_{10}\text{S/P}) + 3.77$$

Data analysis, titre calculation, and the classification of the results can be easily performed by using the FlockSoft™ software. Negative test results are classified into titre group 0, doubtful results into titre group 1, and positive test results into titre groups 2 to 18 depending on the S/P ratio. Please refer to the FlockSoft™ manual for further information.

Evaluation

- **Samples with the S/P ratio < 0.2 are diagnosed as negative.** Specific antibodies to NDV could not be detected.

- **Samples with the S/P ratio ≥ 0.2 and < 0.3 are diagnosed as doubtful.** Doubtful results should be grouped to the majority of the positive or negative results. It is recommended to re-test doubtful results after a few weeks. Doubtful results from recently vaccinated animals may indicate a beginning increase in the formation of specific antibodies. Doubtful results from animals with repeated vaccinations may indicate an insufficient formation or a decrease of specific antibodies.

- **Samples with the S/P ratio ≥ 0.3 are diagnosed as positive.** Specific antibodies to NDV were detected.

Labor Diagnostik Leipzig - Deutscher Platz 5b - 04103 Leipzig - Germany
Phone +49-341-124540 - Fax +49-341-1245460 - info@lab-leipzig.de – www.lab-leipzig.de